Comparison of Two Methods for Anthocyanin Quantification

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Abstract

The pH differential method (AOAC method 2005.02) by spectrophotometry, and high performance liquid chromatography (HPLC) are methods commonly used by researchers and the food industry for quantifying anthocyanins of samples or products. This study was carried out to determine if a relationship exists between these two frequently utilized analytical methods. Seven fruit juice samples, each containing an array of individual anthocyanins, were analyzed by pH differential and HPLC (two different conditions). Additional samples that had been processed in the lab were also included in the comparison ($n > 500$). This study demonstrated a high linear correlation ($r > 0.925$, $p \leq 0.05$) between the pH differential method and HPLC (both systems) when determining the amount of anthocyanins within samples. In both methods, total anthocyanins were greater when values were expressed as malvidin-3-glucoside than as cyanidin-3-glucoside. For laboratories that do not have the capability for HPLC analysis, the pH differential method offers a straightforward and economical method to determine total anthocyanins. This study also demonstrated the importance of reporting the standard used to express the values. Analytical methods used and certified anthocyanin standards are critical for anthocyanin research and the food industry.

INTRODUCTION

Anthocyanins are compounds that give small fruits and their processed products red, blue, and black hues, and are an important indicator of quality (Wrolstad et al., 2005). They degrade during processing and storage, which can have an impact on color and potentially alter the nutritional benefits. Most research on the quantitation, separation, and identification of anthocyanins has relied on expensive equipment like a HPLC. Measured anthocyanin content of a sample can be influenced, in part, by the method used for conducting the analysis (Lee et al., 2005; Lee and Finn, 2007; Dossett et al., 2008; Lee et al., 2008).

The objective of this work was to compare two commonly used anthocyanin determination methods: pH differential (AOAC method 2005.02) and HPLC analysis (two different mobile phase systems), and also to help understand reported anthocyanin values in the literature based upon the method used.

MATERIALS AND METHODS

The pH differential method was conducted as described in detail by Lee et al. (2005) using a spectrophotometer and a plate reader for its high sample throughput. The two HPLC conditions used are described in Lee et al. (2005) and Lee and Finn (2007) regarding mobile phases and analytical columns used (systems 1 and 2, respectively). All samples were expressed as cyanidin-3-glucoside (cyd-glu, molar extinction coefficient of 26,900 $L \cdot cm^{-1} \cdot mol^{-1}$ and molecular weight of 449.2 $g \cdot mol^{-1}$) or malvidin-3-glucoside (mvd-glu, molar extinction coefficient of 28,000 $L \cdot cm^{-1} \cdot mol^{-1}$ and molecular weight of

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463.3 $g \cdot mol^{-1}$) equivalents, two commonly used standards. Purchased standards were used in the HPLC portion of this study. Commercially available juice samples, elderberry samples, black raspberry samples, and 'Cabernet Sauvignon' grape samples were included in the comparison (Fig. 1). Details of the different methods used are described in Lee et al. (2008).

RESULTS AND DISCUSSION

Overall, values acquired from all methods (pH differential and the two different HPLC mobile phase systems) had a high linear correlation with one another for quantifying anthocyanins ($r > 0.925$; Table 1) despite the variation in individual anthocyanins found in the juice samples. HPLC obtained values were higher than anthocyanin values obtained by the pH differential values, except for cranberry juice cocktail expressed as cyd-glu.

All total anthocyanin measurements, regardless of the analytical method, were greater when expressed as mvd-glu, than when expressed as cyd-glu (Table 1). This is due to the difference in extinction coefficient and molecular weight values of mvd-glu and cyd-glu that affect pH differential calculations (Lee et al., 2008). This emphasizes the importance of indicating which individual anthocyanin was used for the calculation. The greater value when analyzed by HPLC, might be due to the difference in the absorbance of the two standards, difference in purity of the purchased standard, and the difference in individual anthocyanins present in these samples (Dossett et al., 2008; Lee et al., 2008).

HPLC is an invaluable tool for identifying and quantifying individual anthocyanin in a sample. However, the pH differential method is a simple, rapid, and an economical means for determining the amount of anthocyanins in a sample. The pH differential method is a good alternative for laboratories that do not have access to a HPLC, and this method has been verified by AOAC's strict validation process (Lee et al., 2005). The use of a microplate reader in place of a spectrophotometer expedites the sample preparation and analysis time of the pH differential method even further. Although accuracy and precision of the microplate reader itself were not tested, the comparison between the cuvette readings to microplate readings were made (Lee et al., 2008) and these values had a high linear correlation ($r = 0.985$, $p \leq 0.05$). These findings demonstrated that a microplate reader is an acceptable alternative to a spectrophotometer to reduce preparation time, reagents usage, and chemical waste when processing samples.

Commercially available fruit juice samples, elderberries (Lee and Finn, 2007), black raspberries (Dossett et al., 2008), along with 'Cabernet Sauvignon' grapes anthocyanin values were compared and the calculated linear correlation was 0.931 ($n > 500$, $p \leq 0.05$; Fig. 1.).

CONCLUSIONS

Depending on the intent of the researcher, only quantitation might be required, though in other cases a more detailed analytical method might be needed when qualitative and quantitative information are important. Comparison of total anthocyanin values among different research groups and literature should be conducted with caution since values reported in the literature were dependent upon the method and standard used. Until a certified anthocyanin standard is available, this study should aid in the ability of different laboratories to compare their values with each other, since values acquired from all methods used in this study (pH differential and two common HPLC solvent systems used) were highly correlated with one another.

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Tables

Table 1. Total anthocyanin content (mg of anthocyanins/100 mL) of all samples, from both HPLC systems and the pH differential method (with cuvettes and microplates).

	Method used							
	pH differential conducted with cuvettes		pH differential conducted with microplates		HPLC system 1		HPLC system 2	
	(expressed as cyd-glu)	(expressed as mvd-glu)	(expressed as cyd-glu)	(expressed as mvd-glu)	(expressed as cyd-glu)	(expressed as mvd-glu)	(expressed as cyd-glu)	(expressed as mvd-glu)
Cranberry juice cocktail	1.31 (0.01)	1.38 (0.01)	1.33 (0.01)	1.40 (0.01)	1.08 (0.11)	1.86 (0.19)	0.92 (0.04)	1.65 (0.07)
Pomegranate juice	5.92 (0.09)	6.25 (0.09)	5.94 (0.03)	6.27 (0.03)	25.19 (2.69)	43.54 (4.65)	23.63 (0.23)	42.60 (0.42)
Blueberry juice	3.56 (0.03)	3.76 (0.04)	3.82 (0.02)	4.04 (0.02)	14.09 (1.37)	24.35 (2.37)	9.97 (0.21)	17.98 (0.37)
Tart cherry juice	2.09 (0.02)	2.20 (0.02)	2.11 (0.04)	2.23 (0.04)	4.14 (0.21)	7.16 (0.37)	4.41 (0.07)	7.96 (0.13)
Grape juice	1.64 (0.04)	1.73 (0.04)	1.60 (0.02)	1.69 (0.02)	4.67 (0.40)	8.08 (0.69)	4.55 (0.06)	8.20 (0.10)
Black cherry juice	4.39 (0.03)	4.64 (0.03)	4.74 (0.02)	5.01 (0.02)	12.54 (0.65)	21.67 (1.12)	11.79 (0.15)	21.25 (0.27)
Concord grape juice	3.12 (0.06)	3.29 (0.07)	3.05 (0.03)	3.22 (0.03)	9.23 (0.56)	15.95 (0.97)	9.18 (0.11)	16.55 (0.19)

Cyd-glu = cyanidin-3-glucoside; mvd-glu = malvidin-3-glucoside; values in parenthesis are standard errors; HPLC system 1 was described in Lee et al., 2005; HPLC system 2 was described in Lee and Finn, 2007.

Figures

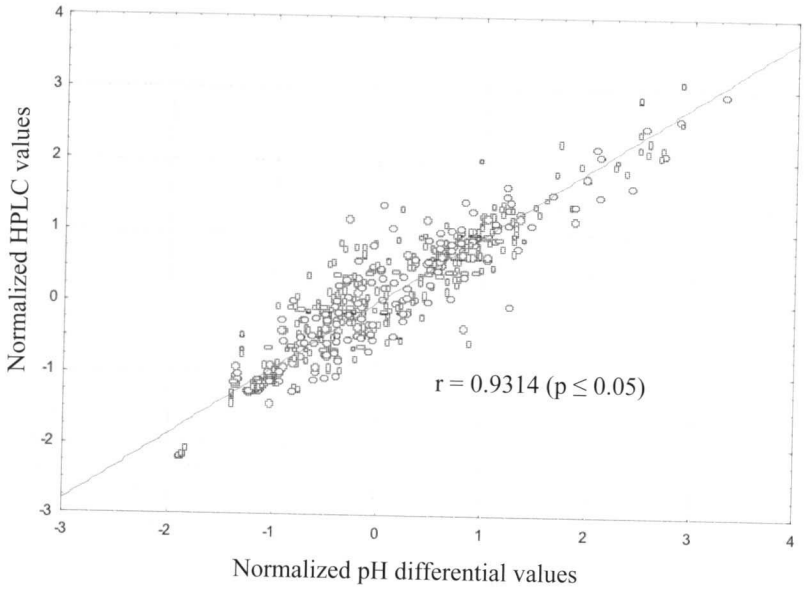

Fig. 1. Normalized High Performance Liquid Chromatography (HPLC) obtained values versus normalized values obtained by the pH differential. Anthocyanin contents were obtained from four studies. Fruit juices, elderberry, black raspberry, and 'Cabernet Sauvignon' grape samples were used (n = 517). 'r' value indicates correlation coefficient.